Institute of Paper Science and Technology
Atlanta, Georgia

IPST Technical Paper Series Number 777

A Simple and Rapid Method to Determine Hexeneuronic Acid Groups in Chemical Pulps

X.S. Chai, J.Y. Zhu, and J. Li

March 1999

Submitted to
10th International Symposium on
Wood and Pulping Chemistry
Yokohama, Japan
June 1999

INSTITUTE OF PAPER SCIENCE AND TECHNOLOGY
PURPOSE AND MISSIONS

The Institute of Paper Science and Technology is an independent graduate school, research organization, and information center for science and technology mainly concerned with manufacture and uses of pulp, paper, paperboard, and other forest products and byproducts. Established in 1929, the Institute provides research and information services to the wood, fiber, and allied industries in a unique partnership between education and business. The Institute is supported by 52 North American companies. The purpose of the Institute is fulfilled through four missions, which are:

- to provide a multidisciplinary education to students who advance the science and technology of the industry and who rise into leadership positions within the industry;
- to conduct and foster research that creates knowledge to satisfy the technological needs of the industry;
- to serve as a key global resource for the acquisition, assessment, and dissemination of industry information, providing critically important information to decision-makers at all levels of the industry; and
- to aggressively seek out technological opportunities and facilitate the transfer and implementation of those technologies in collaboration with industry partners.

ACCREDITATION

The Institute of Paper Science and Technology is accredited by the Commission on Colleges of the Southern Association of Colleges and Schools to award the Master of Science and Doctor of Philosophy degrees.

NOTICE AND DISCLAIMER

The Institute of Paper Science and Technology (IPST) has provided a high standard of professional service and has put forth its best efforts within the time and funds available for this project. The information and conclusions are advisory and are intended only for internal use by any company who may receive this report. Each company must decide for itself the best approach to solving any problems it may have and how, or whether, this reported information should be considered in its approach.

IPST does not recommend particular products, procedures, materials, or service. These are included only in the interest of completeness within a laboratory context and budgetary constraint. Actual products, procedures, materials, and services used may differ and are peculiar to the operations of each company.

In no event shall IPST or its employees and agents have any obligation or liability for damages including, but not limited to, consequential damages arising out of or in connection with any company's use of or inability to use the reported information. IPST provides no warranty or guaranty of results.

A SIMPLE AND RAPID METHOD TO DETERMINE HEXENEURONIC ACID GROUPS IN CHEMICAL PULPS

X.S. Chai, J.Y. Zhu, and J. Li

Institute of Paper Science and Technology
500 10th Street, N.W.
Atlanta, GA 30318, USA
(404) 894-4778 (FAX)
Jian.Li@ipst.edu

ABSTRACT

This paper describes a new procedure to determine HUA in kraft. The method is based on the highly selective hydrolysis of HUA from a pulp sample in a mercuric chloride-sodium acetate solution. By choosing the proper hydrolysis condition (the concentration of hydrolysis agent and the compositions of hydrolysis solution), not only can a complete hydrolysis of HUA from pulps be achieved within 30 minutes, but also the amount of HUA can be directly determined by UV spectrophotometer from the resulting solution. By using dual-wavelength spectroscopic technique (at 260 and 291 nm), the spectral interference from theleached lignin in the resulting solution can be eliminated. The present method is very simple and rapid, and only a very small sample size is required for analysis.

BACKGROUND

The presence of hexeneuronic acid groups (HUA) in kraft pulps has received a lot of attention in the kraft pulp industry recently, because HUA affects the kappa number determination by reaction with permanganate, and can react with certain bleaching chemicals, e.g. chlorine dioxide and ozone, but not some others such as oxygen and peroxide. Therefore, the presence of the HUA increases chlorine dioxide consumption in chlorine dioxide bleaching and overestimates the residual lignin in pulps that causes the over dosage of peroxide in peroxide bleaching.

Several methods for quantitative analysis of HUA in pulps have been reported. Vuorinen et al. presented a method to quantify HUA content in pulp by UV photometric measurement based on an acid hydrolysis of HUA from pulp [1]. Teleman et al. described a method involving an enzymatic hydrolysis using xylanase followed by NMR spectroscopy [2]. The HUA through an acidic or enzymatic hydrolysis released from a pulp can also be separated and determined by an anion exchange chromatography [3] or by using a capillary zone electrophoresis technique [4]. However, all these methods are quite time consuming mainly because of a very low efficiency in either enzymatic or acidic hydrolysis of HUA in pulp. Recently, Gellerstedt and Li [5] developed an HPLC method for determining the HUA in chemical pulps using a combination of hydrolysis using mercuric acetate, peroxide oxidation coupled with thiobarbituric acid to form a colored structure, which can be determined spectroscopically. Although the hydrolysis reaction of HUA pulp in this method is very efficient, it is still complicated and time-consuming due to many experimental procedures involved in quantifying the hydrolysis product.

In this work, we developed a simple and rapid method for spectroscopically quantifying the content of HUA present in chemical pulps. The amounts of HUA in various kraft pulp samples determined using this method are also presented.

EXPERIMENTAL

Apparatus

A diode array spectrophotometer (UV-845x, Hewlett-Packard, CA, USA), equipped with a 10 mm silica cell, was used for the spectrophotometric measurements.

Chemicals and materials

Mercuric chloride ($HgCl_2$) and sodium acetate trihydrate ($CH_3COONa{\cdot}3H_2O$) of analytical grade from commercial sources were used. Deionized water was used in solution preparation. The pulp samples used were from different laboratory batch pulping processes conducted in our laboratory. The hydrolysis solution consists of 22 mM (0.6%) mercuric chloride and 0.7% sodium acetate. A birch pulp sample with known HUA content is used as a standard for calibration. The standard pulp is obtained from the Royal Institute of Technology, Sweden. The HUA content of this standard pulp is 45 μMol/gram measured by Gellerstedt and Li [5] by a HPLC method.

Experimental procedures

Accurately weigh about 0.05 gram of air-dried pulp handsheet with known moisture content and put it in a 20-mL vial, add 10 mL of hydrolysis solution, and seal the vial by a septum. Good mixing of the chemicals in the vial is obtained by hand shaking. The vial is then heated for 30 minutes in a water bath with a temperature range of 60-70°C. The solution is cooled to room temperature, and then measured by spectrophotometer at the wavelengths of 260 and 291nm after filtration.

RESULTS AND DISCUSSIONS

Determination of HUA by UV-spectrophotometry

HUA has an absorption in the UV range due to the unsaturated structures. Therefore, it can be determined by spectroscopic method as long as there

is no spectral interference. Vuorinen et al. used an UV photometric method (at wavelength of 245 and/or 285 nm) to measure furan derivatives that are the further hydrolysis products from HUA in an acidic medium [1]. In this method, the spectral interference in the UV range from the strong absorption of acid-soluble fraction of residual lignins significantly affected the measurements of HUA. Ludwigs et al. [6] found that mercuric salt can hydrolytic release of the unsaturated uronic acid from 2-acetamido-2-deoxy-3-O-(β-D-threo-4-enopyranosyluronic acid)-D-glucose. Since the hydrolysis reaction is also highly selective, no lignin will be released in such pretreatment. Therefore, the interference from lignin in UV spectrophotometric measurement can be eliminated. Gellerstedt and Li [5] used a mercuric acetate buffer solution for the hydrolysis of HUA from pulp because mercuric acetate has a higher hydrolysis efficiency than mercuric chloride. Unfortunately, an extremely high UV absorption from the hydrolysis-buffer solution (blank) makes it impossible to apply a spectrophtometric method for directly measuring HUA in the hydrolysis solution.

In the present study, we use mercuric chloride as a hydrolysis agent because of its relatively low absorptivities in the UV range. Thus, we can easily measure the concentration of HUA in the hydrolysis solution. A typical spectrum of HUA in the given hydrolysis medium (22 mM $HgCl_2$ in 0.7% CH_3COONa solution) is shown in Fig. 1. The absorption at 260 nm agrees with Beer's Law, i.e., the absorption intensity is linearly proportional to the concentration of HUA in the hydrolysis solutions as shown in Fig. 2. The mole absorptivity of HUA at wavelength of 260 nm is 4.25×10^3 $mol^{-1} \cdot L \cdot cm^{-1}$. Therefore, we can simply apply a spectrophotometric method to directly measure the concentration of HUA in the hydrolysis solution. The HUA content in pulp can be calculated as,

$$C_{HUA} = 0.235 \times \frac{A \cdot V}{w} \qquad (1)$$

where C_{HUA} is the content of hexeneuronic acid groups in pulp, μMol per gram; A is the absorbance at 260 nm; V is the volume (mL) of hydrolysis solution; w is the oven dry weight (gram) of dried pulp handsheet. The 0.235 is the calibration factor with the given hydrolysis medium.

The leaching of lignins from pulp during the hydrolysis process

It is well known that a small amount of lignin can be leached from pulp into the solution during hydrolysis processes. Due to very high absorptivity of lignin in the UV range, its effect on HUA determination is significant, especially in analysing pulps with higher kappa numbers (lignin contents), which can result in overestimation of the HUA content of a given pulp using Eqn. (1).

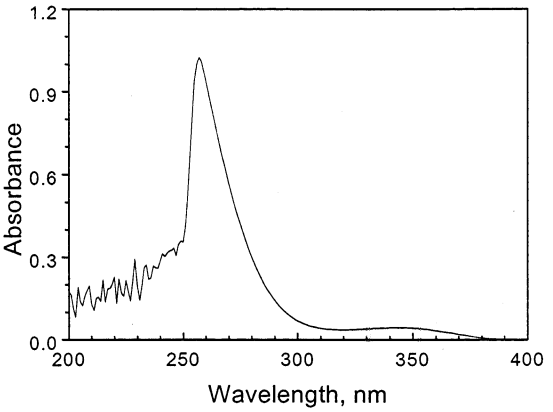

Fig. 1 Spectrum of HUA in a mercuric chloride-sodium acetate solution.

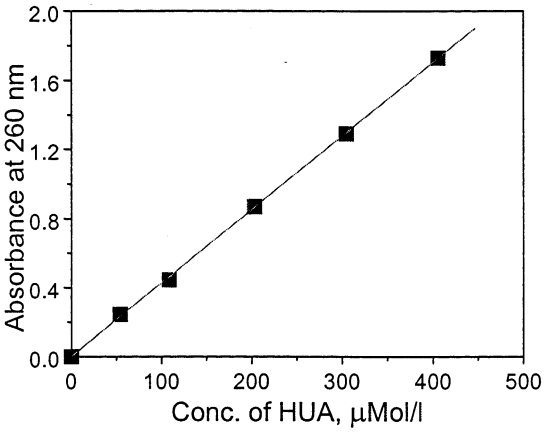

Fig. 2 A calibration curve of HUA.

We found that the spectral intensities of lignin at wavelengths of 260 and 291 nm are equal regardless of the lignin concentrations in the hydrolysis solution as shown in Fig. 3. Therefore, we can use a dual-wavelength correction method in the HUA measurement to correct for the spectral interference from the leached lignin.

The spectral intensities at these two wavelengths can be written as,

$$A_{260} = \varepsilon_{HUA}^{260} \cdot L \cdot C_{HUA} + \varepsilon_{lignin}^{260} \cdot L \cdot C_{lignin} \qquad (2)$$

and

$$A_{291} = \varepsilon_{HUA}^{291} \cdot L \cdot C_{HUA} + \varepsilon_{lignin}^{291} \cdot L \cdot C_{lignin} \qquad (3)$$

respectively. where ε_{HUA}^{260}, $\varepsilon_{lignin}^{260}$, ε_{HUA}^{291}, and $\varepsilon_{lignin}^{291}$ are the molar absoptivities of HUA and lignin at wavelengths of 260 and 291 nm, respectively. L is the volume (liter) of the hydrolysis solution. Because the spectral intensities (or absorbances) of lignin at wavelengths of 260 and 291 nm are the same, and the absorbance of HUA at wavelength of 291 is much

lower than that at 260 nm as shown Fig. 1, for a given spectrum measured in a hydrolysis solution, we can use the spectral intensity at 291 nm (mainly contributed by the lignin) to correct for the spectral contribution by the lignin at wavelength 260nm. Therefore, the concentration of HUA in the hydrolysis solution after spectral correction will be,

$$C = \frac{A_{260} - A_{291}}{(\varepsilon_{HUA}^{260} + \varepsilon_{HUA}^{291}) \cdot L} = K(A_{260} - A_{291}) \quad (5)$$

where, C is the HUA concentration in the hydrolysis solution and K is a constant.

Thus, the HUA content in pulp can be calculated as,

$$C_{HUA} = 0.273 \times \frac{(A_{260} - A_{291}) \cdot V}{w} \quad (6)$$

Eqn.(6) eliminated the spectral interference from lignin. 0.273 is the calibration factor that is obtained using a standard pulp sample.

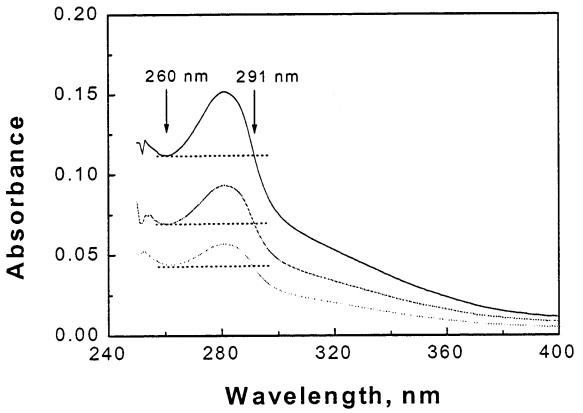

Fig. 3 Typical spectra of lignin in hydrolysis solutions.

The rate of hydrolysis of HUA using mercuric chloride

Both mercuric acetate and mercuric chloride can selectively hydrolyze hexeneuronic acid groups from pulp. It was demonstrated that the hydrolysis rate of HUA from pulp when using mercuric acetate is much higher than using mercuric chloride [5]. However, the solubility of mercuric acetate in water is very low, it is therefore mandatory to dissolve it in a buffer solution as did Gellerstedt and Li in their work. Thus, the strong UV absorption from the hydrolysis medium makes it impossible for a direct spectrophotometric measurement on the hydrolysis solution. Although mercuric chloride is also a highly selective hydrolysis agent for HUA, its hydrolysis rate is relatively low. The study showed that the time for complete hydrolysis of HUA from pulp was relatively long (about 90 min. at 60 °C) [5].

Since mercuric chloride gives a relatively low absorptivity in the UV range, especially at a wavelength longer than 250 nm, a direct UV measurement can be realized when using mercuric chloride as a hydrolysis solution. In this work, our main effort was to increase the hydrolysis rate of mercuric chloride to release HUA from pulp. A higher temperature can increase the hydrolysis of HUA from pulp. Increasing the concentration of mercuric chloride in the solution can also increase the hydrolysis reaction rate. A solution containing 22 mM of mercuric chloride was chosen in this work due to the limit of the solubility of mercuric chloride. The dried pulp sample is directly put into the hydrolysis solution rather than placed into water to swell the pulp as in the previous work [5]. Thus, the dilution of hydrolysis agent can be avoided, and the concentration of the hydrolysis agent is 2 times that used in the previous work [5]. With this condition, a complete hydrolysis of HUA from pulp in this mercuric chloride solution can be achieved within 30 minutes at a temperature of 60°C or higher as shown in Fig. 4. A higher temperature not only increases the rate of hydrolysis of HUA from pulp, but also improves the pulp swelling.

It was found that there is no significant difference in the measured concentration of HUA in the hydrolysis solutions with or without a pre-swelling step in our experiments, the swelling step [5] before the hydrolysis reaction is not necessary. Thus, the experimental procedure used in this study is simpler and more efficient.

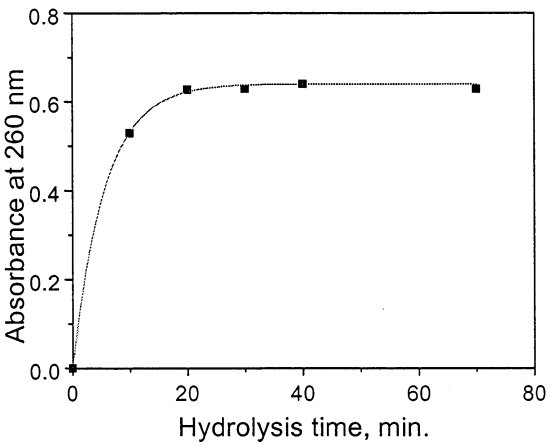

Fig. 4 Release of HUA from pulp in a mercuric chloride-sodium acetate solution at 60°C.

Due to the mercuric chloride solution's stronger acidity, a further degradation of HUA into furan derivatives might take place. By adding a certain amount of sodium acetate into the solution, the pH of the solution can be kept within a neutral range (pH = 6 - 7). Thus, a possible acidic degradation of HUA can be avoided.

The precision of the method.

A repeatability test of the present method was conducted using a birch pulp with 5 different sample sizes. Table I lists the test results. It was found that the relative standard deviation of the 5 measurements was less than 2.0%, indicating that the measurements are very repeatable.

Table 1. Measured HUA content using different amounts of pulp sample.*

Sample No.	Pulp Weight (g)**	A_{260}-A_{291}	HUA, μMol/g pulp
1	0.0498	0.8536	43.8
2	0.0526	0.9395	45.6
3	0.0472	0.8144	44.1
4	0.0490	0.8696	45.3
5	0.0523	0.9319	45.5
Mean			**44.9**
RSTD			**1.9%**

* 10 mL hydrolysis solution was used in the experiment. ** Moisture content of pulp is 8%.

Applications

According to Clayton [7], 4-O-methylglucuronic acid groups in wood xylan can be rapidly hydrolyzed during alkaline pulping to form corresponding unsaturated acid (or HUA) by the loss of methanol. We studied the HUA formation during kraft pulping using the present method (Eqn. (6)). Methanol concentrations in cooking liquors were measured used the method demonstrated in our previous study [8]. The unit of mmol/kg wood is used to characterize HUA and methanol in this study. To convert to this unit, the measured HUA content in each pulp sample is divided by the pulp yield for the given pulping process and the measured methanol concentration in each cooking liquor is divided by the organic solids content for the given liquor. Fig. 5 shows measured HUA from the pulps and methanol from cooking liquors taken from 8 conventional kraft pulping processes. It was found that both the HUA content in pulp and methanol concentration in the cooking liquor increased during the early part of the cooking (the heating up period of the wood). However, as the cooking process proceeds, the HUA content in pulp decreased gradually and the methanol concentration reached a constant value. The reduction of HUA is probably due to the dissolution of xylan in the cooking liquor at the later stage of the pulping process. Analysis of the dissolved xylan fraction present in kraft cooking liquor showed that this fraction contained the HUA [9].

We conducted 7 different pulping processes and measured the HUA content from the pulp samples obtained during the wood chip heating up period. We found that the HUA content is linearly proportional to the methanol concentration in the cooking liquor as shown in Fig. 6. The linear relationship between these two species supports the theory that xylan hydrolysis is the main reaction pathway for the formation of methanol in chemical pulping proposed in our previous study [10]. We also found that the linear line in Fig. 6 has a negative intercept, indicating that some of the low molecular weight xylans were released from wood chips and dissolved into the cooking solution. The HUA in the dissolved xylans cannot be detected by the present method.

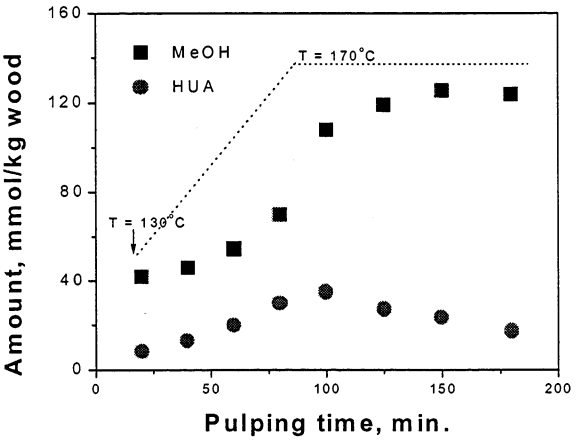

Fig. 5 The content change of HUA in pulp during the kraft pulping process.

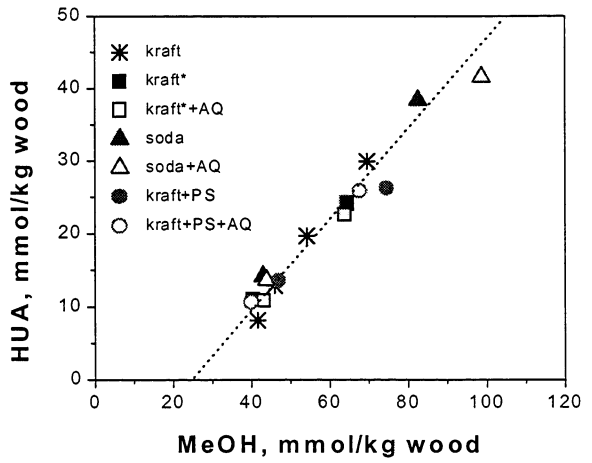

Fig. 6 The relationship between the content of HUA in pulp and methanol formed in the initial stage of the cooking.

A number of pulp samples, including hardwood and softwood, from several laboratory pulping processes were analyzed by the present method to determine the HUA contents. The results are presented in Table II. The first two maple pulps were produced under different effective alkali (EA) concentrations. The low-EA pulp with higher kappa number had a higher HUA content. The same maple pulps after acid hydrolysis still contain about a third of the original amount of HUA. The gum pulp from RDH cooking had 30% lower HUA than that of conventional pulp. It was found that hydrolysis by the

present method has almost no impact on the color of the pulps, nor did it brighten the pulps.

Table II also shows that the HUA contents in all the hardwood pulps are higher than those in the softwood (pine) pulps despite the fact that softwood pulps have higher kappa numbers. This indicates that the softwood pulp has a lower xylan content than hardwood.

Table 2. The HUA contents in different kraft pulps.

Wood Specie	Cook type	Pulp Kappa	HUA μMol/g pulp
Maple	High-EA	16.2	62.3
Maple	Low-EA	18.5	66.2
Gum	RDH	7.8	39.6
Gum	Conventional	11.4	55.7
Birch	High-EA	21.8	69.3
Pine	Kraft	21.3	32.9
Pine	Kraft	28.0	42.2
Pine	Kraft	36.0	46.5
Pine	Kraft	39.2	51.7
Pine	Kraft	43.8	52.3

CONCLUSIONS

A very simple and rapid spectroscopic method to determine hexeneuronic acid groups in pulp has been developed. The method uses concentrated mercuric chloride to obtain increased hydrolysis reaction rate and reduced UV background spectral inference. After a dried pulp is directly hydrolyzed in a solution with proper concentrations of mercuric chloride and sodium acetate, a spectroscopic measurement on the hydrolysis solution can be directly conducted. By using dual-wavelength spectroscopic technique (at 260 and 291 nm), the spectral interference from the leached lignin in the resulting solution can be eliminated. Compared with other available methods, this procedure significantly reduces the experimental time for determining the HUA content in pulp.

ACKNOWLEDGEMENT

This research was partially supported by the United States Department of Energy (Grant No. DE-FC07-96ID13438). Thanks are also due to Dr. Jeibing Li of Royal Institute of Technology, Stockholm, Sweden, for providing us with the standard pulp sample and helpful discussions.

REFERENCES

1. T. Vuorinen, J. Buchert, A. Teleman, M. Tenkanen, P. Fagerstrom, Proc. 1996 Int. Pulp Bleaching Conf., *TAPPI Press*, Atlanta, p.43.
2. A. Teleman, V. Harjunpaa, M. Tenkanen, J. Buchet, T. Huasalo, T. Drakenberg and T. Vuorinen, *Carbohydr. Res.*, 272, 55-71,1995.
3. M. Tenkanen, T. Huasalo, M. Siika-aho, J. Buchet and L. Viikari, *Int. Symp. Wood Pulping Chem. 8th*, Proceedings, Vol. 3, p. 189-194, 1995.
4. O. Dahlman, A. Rydlund, and A. Lindquist, *Carbohydr. Res.*, 294:41, 1996.
5. G. Gellerstedt and J. Li, *Carbohydr. Res.*, 41-51,1996.
6. A. Ludwigs, A. Elgavish, J.D. Esko, E. meezan and L. Roden, *Biochem. J.*, 245, 795-804, 1987.
7. D.W. Clayton, Svensk Papperstidning, 66(4), 115, 1963.
8. X-.S. Chai, B. Dhasmana, and J.Y. Zhu, *J. Pulp and Paper Sci.*, 24(2), 1998.
9. A. Teleman, V. Harjunpaa, M. Tenkanen, J. Buchert, T. Hausalo, T. Drakenberg, and T. Vuorinen, Carbonhydr. Res., 272, 55-71, 1995.
10. J.Y. Zhu, X-.S. Chai, and B. Dhasmana, *J. Pulp and Paper Sci.*, 25(7), 1999 (in press).